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# G Protein Linked Signal Transduction Pathways in Development: Dictyostelium as an Experimental System

## Review

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It has been 22 years since cAMP was identified as the acrasin, i.e., the chemotactic substance mediating aggregation in *Dictyostelium discoideum* (Konijn et al., 1967). cAMP is also known to control gene expression throughout development via cell surface cAMP receptors. Over the last few years, substantial progress has been made in understanding these pathways at a biochemical and molecular level. In this article, we review our present understanding of these mechanisms and compare this system with those controlling similar processes in other eukaryotes.

### cAMP Receptor Mediated Events

*Dictyostelium* grows as single-celled vegetative amoebae. Upon removal of the food source, a multicellular developmental program is initiated. When cells are at a sufficient density, individual cells will initiate a signaling response by secreting cAMP. Two responses are activated in nearby cells: chemotaxis, in which cells move up the cAMP gradient, and signal relay, the synthesis and release of cAMP into the extracellular medium. Both pathways are activated for ~1–2 min before an adaptation process inactivates the responses.

Once cAMP is cleared, both pathways deadapt and, after ~5–6 min, a new wave of cAMP is initiated. These periodic waves of cAMP propagate radially outward while the reacting cells move inwardly toward the aggregating center (see Janssens and van Haastert, 1987). By 10 hr an aggregate of up to  $10^5$  cells is formed. It then differentiates into a migrating slug or pseudoplasmodium containing 20% prestalk cells and 80% prespore cells which are localized in the anterior and posterior of the slug, respectively. The decision to differentiate into either a prestalk or prespore cell and become spatially localized in the slug depends upon the position of the cell in the cell cycle at the moment of starvation (Gomer and Firtel, 1987). By 18 hr culmination begins, and a mature fruiting body is

formed by 24–26 hr. During the multicellular development, cAMP oscillations continue, originating at the tip of the slug or culminating fruiting body, and are important for patterning and morphogenesis (Schaap, 1986).

### Events at the Membrane

The chemotaxis and signal relay pathways are diagrammed in the figure. There are two classes of cAMP receptors, which are defined by the rate of dissociation of cAMP, fast ( $50\text{--}100 \times 10^3/\text{cell}$ ) and slow ( $\sim 4 \times 10^3/\text{cell}$ ). The fast receptors ( $R_A$ ) are probably associated with activation of adenylate cyclase (AC), while the slow receptors ( $R_B$ ) are coupled to the chemotaxis response (Janssens and van Haastert, 1987). Activation of chemotaxis is also reflected in a transient 5- to 10-fold rise in cGMP levels, which peak within 10 s, due to activation and rapid adaptation of guanylate cyclase. Activation of this pathway also results in 2- to 3-fold increases in inositol triphosphate ( $\text{IP}_3$ ) levels within ~5 s, and the addition of 1,4,5- $\text{IP}_3$  to permeabilized cells results in a release of calcium from non-mitochondrial stores (Newell et al., 1988; van Haastert et al., 1989). Both classes of receptors are coupled to G proteins (see below). At present, it is not known if the classes are the products of distinct genes, or whether their different kinetic properties are the result of coupling to different G proteins (or other components of the signaling pathway).

The gene for a cell surface cAMP receptor expressed during aggregation has been cloned (Klein et al., 1988). Expression of the receptor is undetectable in vegetative cells, is maximal during aggregation, and then decreases over the next few hours. This receptor contains seven putative transmembrane domains and a serine-rich carboxyl terminus, structural motifs common to G protein-linked receptors. Ligand occupancy induces phosphorylation of serine residues at the carboxyl terminus (Vaughan and Devreotes, 1988), as is also seen in rhodopsin and the  $\beta$ -adrenergic receptor (Lefkowitz et al., 1988).

An increase in receptor occupancy results in a single transient increase in cGMP or cAMP, lasting a few seconds (cGMP) or minutes (cAMP). Continuous maintenance of the same stimulus elicits no further response. If the cells are then stimulated with a higher concentration of cAMP, they respond proportionally. Repeated responses to incremental increases in cAMP can continue until the receptors are saturated. The sum of the magnitudes of all the responses is equal to the response to a single exposure at a saturating concentration.

Adaptation and receptor phosphorylation are closely associated. After several minutes of continuous occupancy, the receptor is fully phosphorylated and the cells no longer respond to cAMP, although the receptor is still capable of binding cAMP. In vivo, adaptation is reversed by diffusion and hydrolysis of cAMP by developmentally regulated cell surface and secreted forms of phosphodiesterase (PDE; Gerisch, 1987; Kessin, 1988). Removal

of cAMP induces a gradual loss of receptor phosphorylation and a return of the cells to a basal, sensitized state. The presence of continuous high levels of cAMP that saturate PDE, or the addition of nonhydrolyzable forms of cAMP (e.g., cAMPS), results in the down regulation of the receptors and the loss of binding sites on the cell surface (Wang et al., 1988).

### Function of G Proteins in Signaling

Biochemical, genetic, and molecular evidence indicate that cAMP receptor-mediated events are regulated through  $\alpha\beta\gamma$  heterotrimeric G proteins, similar to those found in mammals (Theibert and Devreotes, 1986, 1987; Janssens and van Haastert, 1987; Snaar-Jagalska et al., 1988; Kumagai et al., 1989; Pupillo et al., 1989). In isolated membranes, cAMP binding to the receptor stimulates binding of GTP $\gamma$ S and high-affinity GTPase activity. In addition, GTP $\gamma$ S or GDP $\beta$ S results in a reduction in the affinity of cAMP for the cell surface receptor. These observations are indicative of signal transduction pathways regulated by heterotrimeric G proteins (Gilman, 1987).

Genes for two  $G_\alpha$  protein subunits have been cloned from *Dictyostelium* (Pupillo et al., 1989). These proteins (both ~39 kd) show 45% amino acid sequence identity with each other and with  $G_\alpha$  protein subunits from yeast and mammalian cells. The putative GTP binding/GTPase regions show essentially 100% amino acid sequence identity with those found in other mammalian  $G_\alpha$  proteins. Neither *Dictyostelium* protein has a site for ADP-ribosylation by pertussis toxin.

Some of the functions of the  $G_\alpha$  proteins have been elucidated. Present data suggest that  $G_{\alpha 2}$  is coupled to the chemotaxis receptors that activate phospholipase C (PLC), and is encoded by the *FrigidA* locus (Kesbeke et al., 1988; Snaar-Jagalska et al., 1988; Kumagai et al., 1989). The four mutant alleles in the *FrigidA* complementation group all affect receptor mediated processes and  $G_{\alpha 2}$  protein levels (Lo et al., 1978; Coukell et al., 1983; Kumagai et al., 1989). Two alleles have substantially reduced  $G_{\alpha 2}$  expression. Another is a null mutation due to a 2.2–2.3 kb deletion within the  $G_\alpha$  coding region. The fourth allele (strain HC112) has reduced expression, and preliminary results indicate that it contains a missense mutation in the coding region. All four strains do not aggregate. All except HC112 show no in vivo activation of either the signal relay or chemotaxis pathways, as determined by the activation of guanylate or adenylate cyclase; in HC112, the activation is reduced by 70%–80%. In membranes isolated from two mutant strains, there is a substantial reduction in cAMP-stimulated GTP $\gamma$ S binding and GTPase activity, and the guanine nucleotide-dependent reduction in cAMP affinity is decreased.

Further analysis has shown that either cAMP or GTP $\gamma$ S stimulates the production of 1,4,5-IP $_3$  in permeabilized wild-type cells, but not in *FrigidA* cells (Newell et al., 1988; van Haastert et al., 1989). Although PLC activity has not been directly measured, these and the previously described results suggest that the chemotaxis receptor is coupled to PLC by  $G_{\alpha 2}$  (Janssens and van Haastert,

1987; Newell et al., 1988; Snaar-Jagalska et al., 1988; Kumagai et al., 1989). In *FrigidA* strains, neither the chemotaxis pathway nor the signal relay pathway are activated in vivo, suggesting that  $G_{\alpha 2}$  is at least indirectly linked to AC. However, biochemical studies show that nonhydrolyzable GTP analogs can activate AC in vitro in isolated membranes from both wild-type strains and *FrigidA* strains, which indicates that a G protein other than  $G_{\alpha 2}$  is coupled to AC (Kesbeke et al., 1988; Snaar-Jagalska, 1988). These observations indicate that the chemotaxis and signal relay pathways are interconnected in vivo and that activation of the signal relay pathway is dependent on activation of the chemotaxis pathway. Neither cAMP nor 1,4,5-IP $_3$  alone will activate AC in permeabilized null mutant cells; however, AC is activated if both are applied together; thus, 1,4,5-IP $_3$  may act to link the two pathways (Snaar-Jagalska et al., 1988). The above observations indicate substantive differences with the apparent direct activation of AC by the  $\beta$ -adrenergic receptor and  $G_{\alpha s}$  (Gilman, 1987).

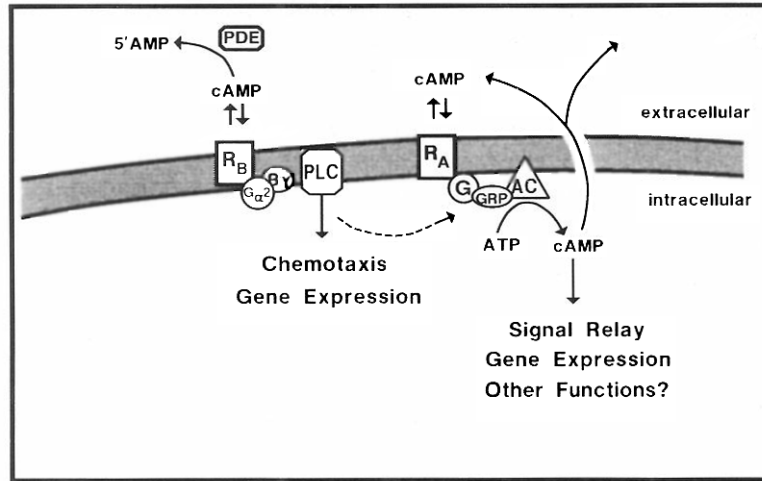
The function of  $G_{\alpha 1}$  has been examined in growing cells and early development by overexpressing it 10- to 20-fold using an actin gene promoter. These cells are very large and have multiple nuclei, suggesting that overexpression of  $G_{\alpha 1}$  affects cytokinesis or some other aspect of cell division. In addition, these cells aggregate and develop poorly (Kumagai et al., 1989).

The timing of expression of  $G_{\alpha 1}$  and AC is not consistent with the hypothesis that  $G_{\alpha 1}$  is the G protein that confers GTP sensitivity to AC. Both the receptor and AC activity are developmentally regulated, peaking during aggregation. In contrast,  $G_{\alpha 1}$  protein is expressed at its highest level during growth. Presumably,  $G_{\alpha 1}$  interacts with some as yet unknown cell surface receptor; the downstream effector enzyme has also not yet been elucidated.

Genetic studies suggest that there are additional components in the *Dictyostelium* pathways. There are four known complementation groups, designated *Synag*, in which the cells do not aggregate because of the inability of cAMP to activate AC. In all of these strains, the chemotaxis pathway is unaffected, and the cells are capable of developing as long as cAMP pulses are supplied either by wild-type cells or by an exogenous source. None of the four mutations directly affect the receptor, AC, or either of the two G proteins that have already been characterized (Theibert and Devreotes, 1986; van Haastert et al., 1987; Kumagai et al., 1989). The biochemical defect in one of these mutants, *Synag7*, has been partially characterized. *Synag7* cells are defective in GTP $\gamma$ S activation of AC, and the defect can be complemented in vitro with GRP, a soluble protein from wild-type cells (Theibert and Devreotes, 1986).

### Regulation of Early Gene Expression by cAMP

Expression of genes essential for aggregation is also induced by cAMP through its cell surface receptor, and thus is tightly linked to chemotaxis and morphogenesis (Mann et al., 1987; Kimmel, 1987; Mann and Firtel, 1988). Pulse-



Model of Membrane Events in Dictyostelium Signal Transduction

The two classes of cAMP receptors shown ( $R_A$  and  $R_B$ ) have been linked to their respective pathways on the basis of kinetic measurements. The dashed arrow indicates that the signal relay pathway is dependent on the chemotaxis pathway. See text for details and appropriate references.

induced genes are not expressed in vegetative cells, can first be detected by 2–3 hr, and are maximally expressed during the peak of aggregation. Repeated stimulation with low concentrations of cAMP induces a precocious and higher level of expression, whereas continuous stimulation, which results in adaptation, substantially reduces or inhibits expression. Analysis of expression of these genes in strains carrying *Synag* or *Frigida* mutations suggests that they are regulated through the chemotaxis pathway (Mann et al., 1988). *Synag* strains show a normal pattern of expression of these genes, indicating that activation of AC and the accompanying rise in intracellular cAMP are not required for expression. In *Frigida* strains, these genes are not expressed and cannot be induced by repeated stimulation with cAMP.

A number of pulse-induced genes encode proteins with functions that are required for aggregation, such as the cAMP receptor itself,  $G_{\alpha 2}$ , contact sites A (a cell adhesion molecule), and gene D2, a serine esterase required for aggregation, as determined by blocking its expression with antisense RNA (Gerisch, 1987; Kimmel, 1987; Klein et al., 1988; Kumagai et al., 1989; Mann et al., 1988). About 100 genes not required for growth are required for aggregation. We expect that a substantial number of these genes are regulated in a manner similar to the initial set characterized.

A paradox exists: how are genes like the receptor induced by repeated stimulation with cAMP, if the gene products are required for the signal transduction pathway? The receptor,  $G_{\alpha 2}$ , and D2 all show a low level of basal expression in *Synag* mutants that lack cAMP signaling. This suggests that initial expression of these genes, presumably sufficient to allow the establishment of a signal transduction system, does not require the oscillatory cAMP signals. From a number of studies, it is believed that a factor in conditioned medium (CMF) may be required for this function. CMF appears to be required to sense the concentration of cells; it can complement the inability of cells at too low a density to develop and express the pulse-induced genes (Mehdy and Firtel, 1985; Gomer et al., 1986; Mann and Firtel, 1989).

In addition to the pulse-induced genes, there are two classes of pulse-repressed genes, whose expression during the preaggregation stage is down-regulated by repeated stimulation with cAMP. The first class is induced upon starvation, maximally expressed at ~2.5 hr of development, and then repressed during aggregation (Mann et al., 1987; Mann and Firtel, 1988). In cell culture systems, the genes can be precociously repressed by pulses of cAMP. The pattern of expression of one of these genes (K5) in *Synag* and *Frigida* strains is opposite that of the pulse-induced gene. It is developmentally induced and continues to be expressed in *Synag* mutants in the absence of exogenous cAMP, but is repressed when cells are given exogenous cAMP pulses. The gene is substantially overexpressed in *Frigida* mutant strains; however, this expression cannot be affected by pulses of cAMP, consistent with the view that the chemotaxis pathway is involved in the repression of these genes.

A second class of cAMP pulse-repressed genes appears to require intracellular cAMP (Kimmel and Saxe, 1986; Kimmel, 1987). For example, gene M4-1 is expressed in vegetative cells, and, during normal development, is repressed during aggregation. It can be precociously repressed in wild-type cells by pulsing with cAMP. In contrast to K5, M4-1 is not repressed by cAMP pulses in *Synag* mutants. This suggests that its repression may require a rise in intracellular cAMP, and thus may be mediated through the signal relay receptors ( $R_A$ ) and cAMP-dependent protein kinase.

In addition to the above genes, the genes encoding extracellular PDE and the inhibitor of extracellular PDE (PDI) are both cAMP-regulated (Coukell and Cameron, 1987; Kessin, 1988). The PDI protein inhibits PDE activity by binding PDE and raising the  $K_m$  of PDE for cAMP. In vivo, this complex is probably irreversible. PDE expression is induced by both pulses and continuously elevated levels of cAMP; PDI shows the opposite regulation. Presumably, the opposing regulatory pathways allow the organism to buffer the extracellular cAMP; high PDE activity is present at high cAMP concentrations, allowing rapid clearing of the ligand. At moderate cAMP levels, the PDE is as-

sociated with the cell surface; at higher levels, it is secreted into the medium. At the present time, the pathways controlling the expression of these two genes have not been fully delineated.

### Control of Late Gene Expression by cAMP

Genes that are preferentially expressed in the early aggregate and in the prestalk region of slugs (prestalk genes) and those preferentially expressed in the posterior of the migrating slug (prespore genes) are also regulated by cAMP. Experiments using the *Synag* mutants, pharmacological agents such as caffeine, and cAMP analogs indicate that both prestalk and prespore genes are regulated through cell surface cAMP receptors (Mehdy and Firtel, 1985; Schaap and van Driel, 1985; Gomer et al., 1986; Schaap et al., 1986; Kimmel, 1987). Like the early pulse-induced genes, these genes are regulated by signal transduction pathways that do not involve a rise in intracellular cAMP. Both classes of late genes are induced at high levels in permeabilized cells when treated with DAG and 1,4,5-IP<sub>3</sub> (Ginsberg and Kimmel, 1989), which suggests that protein kinase C and/or Ca<sup>2+</sup> may be involved in regulating the expression of these genes. In contrast to the pulse-induced genes, the prestalk and prespore genes are preferentially induced by higher, constant levels of cAMP, which result in adaptation of the early classes of receptors. The above results, combined with the fact that the early receptors are present at substantially reduced levels late in development (Klein et al., 1987), suggests that a novel class of cAMP receptors preferentially expressed later in development may be involved in controlling the expression of these genes. Presumably, either G<sub>q</sub>2 or a novel G<sub>q</sub> expressed later in development mediates these events.

### Other Signal Transduction Pathways

While cAMP represents an essential messenger for regulating chemotaxis, gene expression, and cell differentiation throughout aggregation and multicellular stages, there are also other signaling pathways that are beginning to be understood. During growth and the early preaggregation stages, folic acid is also a chemotactic signal and is believed to be used by amoebae to sense bacteria, the food source in the wild (De Wit and Bulgakov, 1986; Segall et al., 1989). There are two classes of folic acid receptors, which can be differentially activated by different folic acid analogs. One class is coupled to AC, while the other is coupled to chemotaxis. Analysis of *Frigida* mutants indicates that G<sub>q</sub>2 is not important in the folic acid response. However, in vitro biochemical data suggest that the folic acid signaling pathway is regulated by G proteins. Thus, there may be additional G<sub>q</sub> subunits involved in folic acid and other signaling systems.

Later in development, adenosine, which is produced from cAMP by PDE and 5' nucleotidase (secreted from cells in the slug stage), appears to be involved in the regulation of patterning and to modulate the signal relay pathway (Schaap and Wang, 1986). The morphogen DIF,

whose structure has recently been identified, and NH<sub>4</sub><sup>+</sup> are also important in controlling prespore and prestalk cell differentiation (Morris et al., 1987; Williams et al., 1987; Bonner et al., 1989; Wang and Schaap, 1989). The mechanism of action of these molecules is not yet understood, but along with cAMP, they are clearly involved in regulating differentiation during the culmination stage and formation of the fruiting body.

### Conclusions

Recent studies in *Dictyostelium* suggest that common signal transduction pathways regulate chemotaxis, aggregation, and gene expression during the development of a multicellular organism. The components of these pathways are those previously found in association with sensory systems: cell surface receptors, G proteins, PLC, and AC. These signal transduction pathways not only control the response of an individual cell to a hormone or neurotransmitter as in mammals, but they can also regulate multicellular developmental pathways that control the differentiation of *Dictyostelium*.

The chemotaxis pathway, in which receptors are believed to activate PLC and downstream pathways, may be analogous to those in sensory systems. While the paradigm of receptor mediated activation of AC was developed in mammalian systems, the requirement for other components, as determined by mutational analysis and the dependence on IP<sub>3</sub>, suggests that the signal relay pathway in *Dictyostelium* differs from the standard R-G-AC pathways. Moreover, it is clear that the chemotaxis and signal relay pathways interact: the signal relay pathway, involving the production of cAMP, becomes secondary to the chemotaxis pathway, i.e., the master controller. This keeps the two pathways tightly coupled, which is probably crucial for aggregation. However, without the cAMP signaling, the entire developmental program does not progress.

The ability to combine molecular approaches, biochemistry, cell biology, and developmental mutants makes this system very amenable to studying regulation of developmental programs by signal transduction pathways. It will be interesting to learn whether these themes are generally found in developing organisms.

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